

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: OKAMURA=6

In re Application of:	)	Confirmation No.: 2935
	)	
Hiroshi OKAMURA et al	)	Art Unit: 1634
	)	
Filed: January 14, 2005	)	Examiner: K. N. Bhat
	)	
Appln. No.: 10/521,176	)	Washington, D.C.
	)	
For: SOLID SUPPORT HAVING	)	
ELECTROSTATIC LAYER AND	)	

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for Patents  
U.S. Patent and Trademark Office  
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Randolph Building, Mail Stop AF  
401 Dulany Street  
Alexandria, VA 22314

Sir:

I, Hirofumi YAMANO, hereby declare that I am one of the inventors of the subject application and experiments were conducted under my direction and control as follows:

**Solid Substrate used in the present invention**

Si/DLC (Diamond Like Carbon)

**Porous substrate**

Si/Ceramic [Pb(ZrTi)O<sub>3</sub>]

Ceramic [Pb(ZrTi)O<sub>3</sub>]: Fuji Ceramics Corporation

Pore size: more than about 10 μm (visible size under SEM)

**Mao et al.**

In paragraph 0051, Mao states that solid and porous substrates can be made from a variety of materials, such as, but no limited to, metals (e.g., Cu, Ag, Au, Al, Zn and Fe): alloys; glasses; **ceramics**; carbon black, silica, silicon; and polymeric materials or plastics. As used herein “porous substrate” refers to a material or a substrate that is a surface with one or more pores or a surface that is uneven, undulating, or not smooth or flat, such as a woven, non-woven, compressed, perforated, or etched material or substrate.

**DNA immobilization by Spotting Method**

- (a) A DLC layer was formed at a thickness of about 25 nm on a silicon substrate cut into 3 mm squares by the ionization deposition method, at an accelerating voltage of 0.5 kV, using a mixed gas of 95% by volume of methane gas and 5% by volume of hydrogen gas as material.
- (b) Next, the methane gas and hydrogen gas were replaced with ammonia gas atmosphere, and aminization was conducted for ten minutes by the plasma method.
- (c) Polyacrylic acid (a polyvalent carboxylic acid) was deposited on the amino group introduced into the surface-treated layer, and the substrate was activated by dipping into an activation solution, in which 0.1 M 1-[3-(diethylamino)propyl]-3-ethylcarbodiimide and 20 mM N-hydroxysuccinimide had been dissolved in 0.1 M phosphate buffer (pH 6) for 30 minutes.

- (d) Then, 5  $\mu$ M Cy3-labeled oligonucleotide (22 mer) was spotted on the substrate using a microarray maker. Then, after it was heated in an oven at 80[deg.] C. for 1 hour, it was immersed and cleaned with 2\*SSC/0.2% SDS for 15 minutes while being stirred by a stirrer.
- (e) Further, in the same manner, it was immersed and cleaned with 2\*SSC/0.2% SDS over 95[deg.] C. for 5 minutes while being stirred by a stirrer, and then dried after rinsing with sterile water.

As a comparison, a porous substrate was treated by the same method through steps (b) to (e) above.

Fluorescence derived from Cy3-labeled oligonucleotide immobilized to the solid support was measured by using FLA 8000 (manufactured by Fujifilm Corporation), fluorescence signals were detected as shown in Figure 1.

As shown in Figure 1, spot signals of the porous substrate lapped over considerably, so that it was difficult to use the porous substrate as a DNA chip.

Additionally, because of the porosity of the porous material, the blocking time took much longer than that of the solid substrate claimed in the instant application. The blocking result of the porous substrate did not satisfy the required level of uniformity, as did the solid substrate.

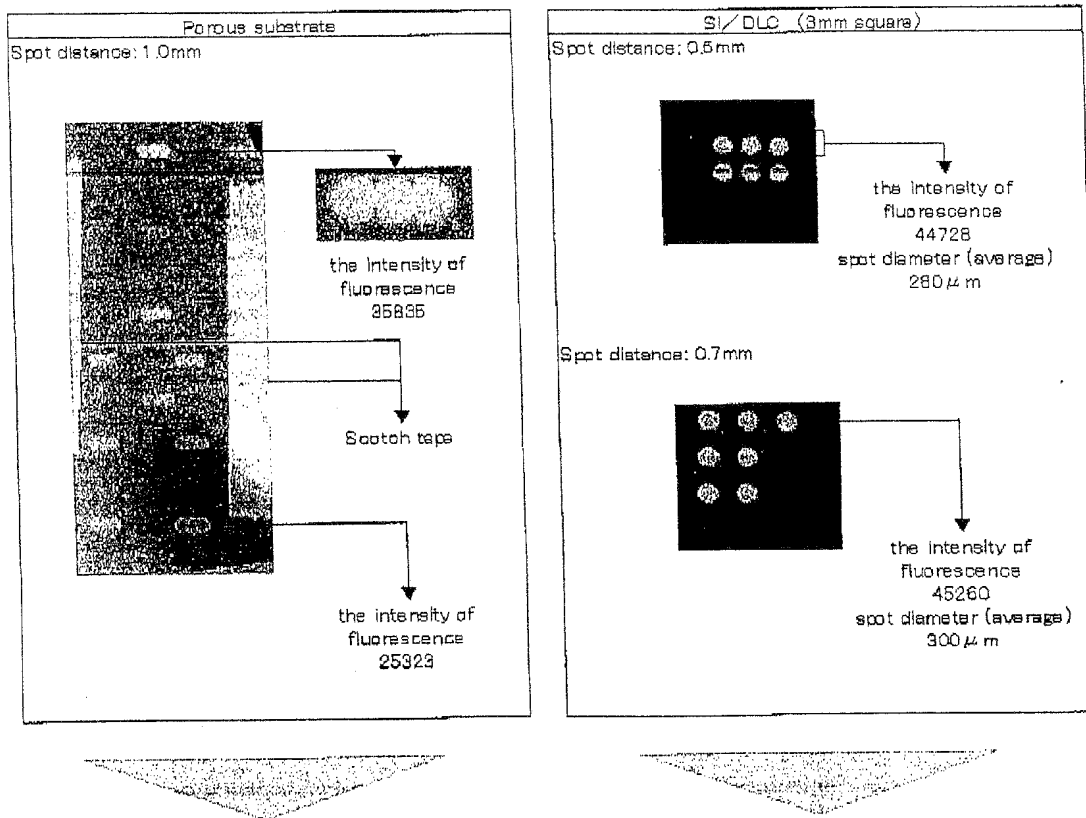
I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

May 10, 2010  
Date

Hirofumi Yamano  
Hirofumi YAMANO

Fig. 1



- Because of the porous material and the roughness of the surface, spot signals lapped over considerably and could not be detected individually in spite of the enough spot distance.
- Therefore, porous material is not acceptable for accurate measurement such as DNA chip.

- Despite narrowly-spaced spot distance, spot signals were detected clearly.